Attorney's Docket No.: 12674-005001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lu-Yieng Liu et al
Serial No. : 10/025,137
Filed : December 19, 2001
Title : METHOD FOR DETECTING ESCHERICHIA COLI

Art Unit : 1634
Examiner : Jehanne E. Souaya

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION BY CHI-HORNG BAIR UNDER 37 C.F.R. 1.132

I, Chi-Horng Bair, hereby declare that:

1. I am the head of Molecular Biology Department at DR. Chip Biotechnology Inc. The subject matter described and claimed in the above-identified application relates to specific nucleic acid sequences for detecting *Escherichia Coli*.

2. In a final Office Action dated August 30, 2004, the Examiner rejected claims 1-3, 5, 6, 8-15, and 36-39 for obviousness rejection over GenBank Accession No. AE005490, GenBank Accession No. AE000346, GenBank Accession No. Z70523, and GenBank Accession No. D90887, in view of Buck et al. Biotechniques, 1999, 27(3): 528-536 ("Buck"), U.S. Patent 5,374,718 to Hammond et al. ("Hammond"), U.S. Patent 5,693,469 to Hogan ("Hogan"), and Tijhie et al., J. Micobiol. Meth. Vol. 18, pp 137-150, 1993 ("Tijhie"). According to the Examiner, (i) the 4 GenBank Accession Nos teach sequences that cover the primer/probe SEQ ID NOs recited in the rejected claims; (ii) Hommond and Tijhie teach picking primers or probes for detection of *Chlamydia pneumonia*, (iii) Hogan teaches targeting sequences within the *E. coli* genome for detections of *E. coli*, and (iv) Buck supports that all nucleic acids selected from the prior art sequences would be expected to function as primers. Then, the Examiner proceeded to

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

December 30, 2004
Date of Deposit

Signature

DEBORAH R. WAST
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conclude that it would be obvious to one skilled in the art to combine all of the cited references and to select PCR primers from the prior art sequences to make the claimed primers.

3. I or others have synthesized a pair of PCR primers, EC-23 and EC-24, that contain sequences selected from *E. coli* genome (GenBank Accession No.: AF319597) based on the same strategy for selecting primers N-1 and N-2 (SEQ ID NOs: 3 and 4, respectively). See page 3, lines 8-25 of the instant application. Summarized in Table 1 below are the sequences of EC-23, EC-24, N1, and N2, as well as their characteristics.

Table 1 The characteristics of adenovirus primer for PCR

Target Gene	Name	Sequence 5' →3'	length	Tm	G+C %	location	Accession No
Epsilon (eaeA) Gene	EC-23	5'-CCCGAATTCGGCACAAGCATAAGC-3'	24	59	54	1-24	AF319597
	EC-24	5'-GTATTCGCCACCAATACCTAAAC-3'	25	55	43	863-840	
N	N-1	5'-TGAATGCGCAAGCTGAAAAAGTAG-3'	24	54	42	82568-82591	AP002562
	N-2	5'-ACGCCGTTAGGTGTATTGATTGTG-3'	24	56	46	83052-83075	

The two pairs of primers, i.e., EC-23/EC-24 and N1/N2, were used respectively to amplify the corresponding target genes from nucleic acid samples of *E. coli* subtypes H, I, A, T, and Non-pathogenic, as well as 6 negative-control microbes: *Staphylococcus aureus*, *Samonella typhi*, *Streptococcus agalactiae*, *Bacillus cereus*, *Shigella dyneria*, and *Listeria monocytogenes*. The amplifications were conducted in the same manner descried in Examples 1 and 2 of the specification, using the following thermal profile: 5 minutes at 95°C, 30 cycles at 95°C for 30 seconds, at 55°C for 30 seconds, 72°C for 30 seconds, the last cycle for 10 minutes at 72 °C. PCR products were analyzed by electrophoresis.

It was found that the N1/N2 primer pair amplified a predicted 500-base pair (bp) specific product from each of the 5 *E. coli* subtypes. In contrast, the EC-23/EC-24 primer pair failed to amplify a predicted 863-bp product. Neither primer pair amplified any product from the 6 negative control microbes.

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4. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

Date: Dec. 14, 2004

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